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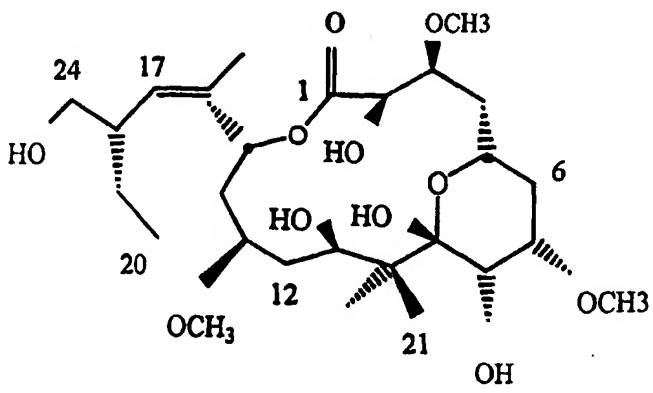


(I)

(57) Abstract: This invention relates to a bioactive compound of formula (I) and to compositions which contain it. In particular, this compound has cytotoxic properties and therefore has utility *inter alia* anti-tumour treatments. The compound was isolated from a marine sponge of the genus *Mycale*.

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# BIOACTIVE COMPOUND

This invention relates to a bioactive compound and to compositions which contain it. In particular, it relates to a compound which has cytotoxic properties and which therefore has utility *inter alia* anti-tumour treatments.

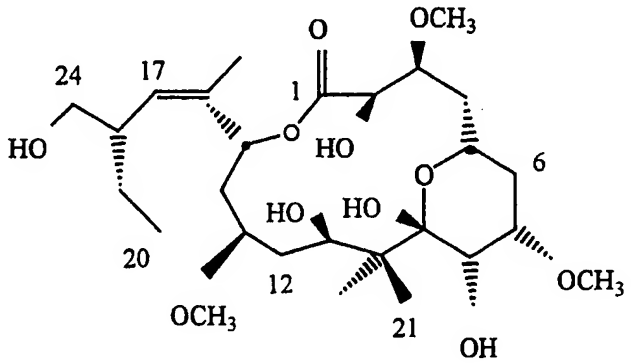
## BACKGROUND

Marine sponges of the genus *Mycale (Carmea)* are a rich source of bioactive secondary metabolites of diverse structures. The mycalysines, mycalolides, deoxytedanolide and the macrolide pateamine have all been isolated from members of this genus and exhibit a variety of properties, including cytotoxic properties. See, for example, Perry *et al.*, *J. Am. Chem. Soc.* (1988), 110, 4850-4851 and Northcote *et al.*, *Tetrahedron Letters* (1991), 32, 6411-6414.

The applicants have now identified a further bioactive compound from a marine sponge of the genus *Mycale*. It is towards this compound, which the applicants have termed Peloruside A, that the present invention is broadly directed.

## SUMMARY OF THE INVENTION

In a first aspect, the invention therefore provides a compound of formula (I)




or a functionally equivalent analogue thereof.

In a further aspect, the invention provides a bioactive compound which has the NMR and/or IR spectral signature of Figures 1 and 2.

In a further aspect, the invention provides a composition which comprises a bioactive compound as defined above, together with a suitable carrier therefor.

Preferably, the composition is a pharmaceutical composition.

In yet a further aspect, the invention provides the use of a bioactive compound as defined above in the preparation of a medicament.

Preferably, said medicament is a cytotoxic medicament, particularly a cytotoxic medicament suitable for use in anti-tumour treatment.

In still a further aspect, the invention provides a method of prophylaxis or therapy which comprises the step of administering to a patient in need of the same a bioactive compound as defined above or a pharmaceutical composition as defined above.

The preferred method is a treatment of a patient against cancer.

**DESCRIPTION OF THE DRAWINGS**

While the invention is broadly as described above, it will also be appreciated that it is not limited thereto but also includes embodiments of which the following description provides examples. In particular, a better understanding of the present invention will be gained through reference to the accompanying drawings in which:

Figure 1 shows the $^{1}H$ NMR spectral signature for Peloruside A; and

Figure 2 shows the IR spectral signature for Peloruside A.

**DESCRIPTION OF THE INVENTION**

As described above, the present invention has as its primary focus a new bioactive compound. This compound has been isolated from a marine sponge of the genus *Mycale* from Pelorus Sound, New Zealand. It has also been found, *inter alia*, to have cytotoxic properties; hence the name Peloruside A.

The compound of the invention can be isolated from marine sponges obtained from New Zealand coastal waters (including Pelorus Sound, Half Moon bay, Stewart Island and Kapiti). The sponges are of a species which belongs to the genus Mycale (Family Mycalidae, Order Poecilosclerida). Individuals of this species may be encrusting or massive, with a chocolate brown ectosome, often with a purple tinge. The sponge surface often has large oscules (2-4 mm diameter) and may appear stippled due to the presence of polychaete worm tubes. The choanosome is light brown with a reticulate skeleton composed of tracts of subtylostyles (220-270 mm long) interspersed with microscleres: anisochelae of 2 size classes, 18-20 and 26-30 mm; sigmas, 20-26 mm; and raphides. The skeleton at ectosome consists of spicules identical to the choanosome, but tangentially arranged and supported by erect spicule brushes.

Sponge specimens which contain Peloruside A can be readily collected by SCUBA, generally at depths of 3 to 20 metres, during the winter months.

Such sponges can be farmed commercially should this prove desirable.

A variety of methods can be used to isolate and purify Peloruside A from samples of *Mycale*, including solvent extraction, partition chromatography, silica gel chromatography, liquid-liquid distribution in a Craig apparatus, adsorption on resins, and crystallisation from solvents.

The isolation and purification methods chosen can be monitored at each step by performing *in vitro* and/or *in vivo* antitumour tests as described by R I Geran, N H Greenberg, M M MacDonald, A M Schumacher and B S Abbott in *Cancer Chemother. Rep.* Part 3, Vol. 3 (2): 1-103 (1972); and by Schmidt, J M, Pettit, G R, in *Experientia* 1978, 34: 659-660. Such tests include the determination of the concentration of active material required to inhibit the growth of tumour cells in culture (eg. the concentration required to inhibit growth by 50 percent or the $E.D._{50}$) and of the dose of active material required to prolong the life of mice bearing transplanted tumours.

A preferred extraction process is described in the experimental section set out hereinafter.

Peloruside A has the structure set out in formula (I) above. However, analogues and/or structural variants of Peloruside A which retain substantially equivalent bioactivity to Peloruside A are contemplated. For example, any of the accessible OH

groups shown in the formula can be replaced with, for example, alkyl groups provided that the poly-oxygenation of the molecule overall is not significantly reduced. Equally, the methoxy groups can be replaced with OH groups or longer chain alkoxy groups.

The selection of substituent groups and the processes by which their substitution can be achieved will be a matter of routine choice for the skilled worker in this field.

Further variations target the alkene side chain, with the length of the chain being altered.

However, such analogues will retain the macrolide structure, inclusive of the pyranose ring and gem-dimethyls as shown in Formula (I).

The fact that Peloruside A has free hydroxyl and replaceable acyl groups also means that acyl esters can be prepared. Such acyl esters of Peloruside A can be prepared by methods well known to those skilled in the art. Acyl derivatives of Peloruside A can be used for the same biological purposes as the parent compound.

Acids which can be used in the acylation of Peloruside A include:

(a) saturated or unsaturated, straight or branched chain aliphatic carboxylic acids, for example, acetic, propionic, butyric, isobutyric, *tert*-butylacetic, valeric, isovaleric, caproic, caprylic, decanoic, dodecanoic, lauric, tridecanoic, myristic, pentadecanoic, palmitic, margaric, stearic, acrylic, crotonic, undecylenic, oleic, hexynoic, heptynoic or octynoic acid;

(b) saturated or unsaturated, alicyclic carboxylic acids, for example, cyclobutanecarboxylic acid, cyclopentanecarboxylic acid, cyclopentenecarboxylic acid, methylcyclopentenecarboxylic acid, cyclohexanecarboxylic acid, dimethylcyclohexanecarboxylic acid or dipropylcyclohexanecarboxylic acid;

(c) saturated or unsaturated, alicyclic aliphatic carboxylic acids, for example, cyclopentaneacetic acid, cyclopentangepropionic acid, cyclohexaneacetic acid, cyclohexanebutyric acid or methylcyclohexaneacetic acid;

(d) aromatic carboxylic acids, for example, benzoic acid, toluic acid, naphthoic acid, ethylbenzoic acid, isobutylbenzoic acid or methylbutylbenzoic acid; and

(e) aromatic-aliphatic carboxylic acids, for example, phenylacetic acid, phenylpropionic acid, phenylvaleric acid, cinnamic acid, phenylpropiolic acid and naphthylacetic acid, and the like. Suitable halo-, nitro-, hydroxy-, keto-, amino-, cyano-, thiocyano-, and lower alkoxyhydrocarbon carboxylic acids include hydrocarboncarboxylic acids as given above which are substituted by one or more of halogen, nitro, hydroxy, keto, amino, cyano, or thiocyano, or loweralkoxy, advantageously loweralkoxy of not more than six carbon atoms, for example, methoxy, ethoxy, propoxy, butoxy, amyloxy, hexyloxy, and isomeric forms thereof.

As described below, Peloruside A has been determined to have cytotoxic properties in tests which are predictive of cytotoxic (including anti-tumour) activity in mammals, including humans. Such properties therefore render Peloruside A suitable for use, alone or together with other active agents, in a number of therapeutic applications, including in anti-tumour treatments.

The administration of Peloruside A is particularly useful for treating animals or humans bearing a neoplastic disease, for example, acute myelocytic leukemia, acute lymphocytic leukemia, malignant melanoma, adenocarcinoma of the lung, neuroblastoma, small cell carcinoma of the lung, breast carcinoma, colon carcinoma, ovarian carcinoma, bladder carcinoma, and the like.

The dosage administered will be dependent upon the identity of the neoplastic disease, the type of host involved, age, health, weight, kind of concurrent treatment, if any, frequency of treatment and therapeutic ratio.

Illustratively, dosage levels of the administered active ingredients can be: intravenous, 0.1 to about 200 mg/kg; intraperitoneal, 1 to about 500 mg/kg; subcutaneous, 1 to about 500 mg/kg; intramuscular, 1 to about 500 mg/kg; orally, 5 to about 1000 mg/kg; intranasal instillation, 5 to about 1000 mg/kg; and aerosol, 5 to about 1000 mg/kg of animal (body) weight.

Expressed in terms of concentration, an active ingredient can be present in the compositions of the present invention for localised use about the cutis, intranasally, pharyngolaryngeally, bronchially, broncholially, intravaginally, rectally, or ocularly in a concentration of from about 0.01 to about 50% w/w of the composition; preferably about 1 to about 20% w/w of the composition; and for parenteral use in a concentration of from about 0.05 to about 50% w/v of the composition and preferably from about 5 to about 20% w/v.

The compositions of the present invention are preferably presented for administration to humans and animals in unit dosage forms, such as tablets, capsules, pills, powders, granules, suppositories, sterile parenteral solutions or suspensions, sterile non-parenteral solutions or suspensions, and oral solutions or suspensions and the like, containing suitable quantities of an active ingredient.

For oral administration either solid or fluid unit dosage forms can be prepared.

Powders are prepared quite simply by comminuting the active ingredient to a suitably fine size and mixing with a similarly comminuted diluent. The diluent can be an edible carbohydrate material such as lactose or starch. Advantageously, a sweetening agent or sugar is present as well as a flavouring oil.

Capsules are produced by preparing a powder mixture as hereinbefore described and filling into formed gelatin sheaths. Advantageously, as an adjuvant to the filling operation, a lubricant such as a talc, magnesium stearate, calcium stearate and the like is added to the powder mixture before the filling operation.

Soft gelatin capsules are prepared by machine encapsulation of a slurry of active ingredients with an acceptable vegetable oil, light liquid petrolatum or other inert oil or triglyceride.

Tablets are made by preparing a powder mixture, granulating or slugging, adding a lubricant and pressing into tablets. The powder mixture is prepared by mixing an active ingredient, suitably comminuted, with a diluent or base such as starch, lactose, kaolin, dicalcium phosphate and the like. The powder mixture can be granulated by wetting with a binder such as corn syrup, gelatin solution, methylcellulose solution or acacia mucilage and forcing through a screen. As an alternative to granulating, the powder mixture can be slugged, i.e., run through the tablet machine and the resulting

imperfectly formed tablets broken into pieces (slugs). The slugs can be lubricated to prevent sticking to the tablet-forming dies by means of the addition of stearic acid, a stearic salt, talc or mineral oil. The lubricated mixture is then compressed into tablets.

Advantageously the tablet can be provided with a protective coating consisting of a sealing coat or enteric coat of shellac, a coating of sugar and methylcellulose and polish coating of carnauba wax.

Fluid unit dosage forms for oral administration such as syrups, elixirs and suspensions can be prepared wherein each teaspoonful of composition contains a predetermined amount of active ingredient for administration. The water-soluble forms can be dissolved in an aqueous vehicle together with sugar, flavouring agents and preservatives to form a syrup. An elixir is prepared by using a hydroalcoholic vehicle with suitable sweeteners together with a flavouring agent. Suspensions can be prepared of the insoluble forms with a suitable vehicle with the aid of a suspending agent such as acacia, tragacanth, methylcellulose.

For parenteral administration, fluid unit dosage forms are prepared utilising an active ingredient and a sterile vehicle with water being preferred. The active ingredient, depending on the form and concentration used, can be either suspended or dissolved in the vehicle. In preparing solutions the water-soluble active ingredient can be dissolved in water for injection and filter sterilised before filling into a suitable vial or ampoule and sealing. Advantageously, adjuvants such as a local anesthetic, preservative and buffering agents can be dissolved in the vehicle. Parenteral suspensions are prepared in substantially the same manner except that an active ingredient is suspended in the vehicle instead of being dissolved and sterilisation cannot be accomplished by filtration. The active ingredient can be sterilised by exposure to ethylene oxide before suspending in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in the composition to facilitate uniform distribution of the active ingredient.

In addition to oral and parenteral administration, the rectal and vaginal routes can be utilised. An active ingredient can be administered by means of a suppository. A vehicle which has a melting point at about body temperature or one that is readily soluble can be utilised. For example, cocoa butter and various polyethylene glycols (Carbowaxes) can serve as the vehicle.

For intranasal instillation, fluid unit dosage forms are prepared utilising an active ingredient and a suitable pharmaceutical vehicle, water being preferred, or by dry powder for insufflation.

For use as aerosols the active ingredients can be packaged in a pressurised aerosol container together with a gaseous or liquefied propellant, for example, dichlorodifluoromethane, carbon dioxide, nitrogen, propane, and the like, with the usual adjuvants such as cosolvents and wetting agents, as may be necessary or desirable.

The invention will now be described with reference to the following experimental section. It will be appreciated that this experimental section is provided by way of illustration of the invention only and is not intended in any way to be limiting.

**EXPERIMENTAL**

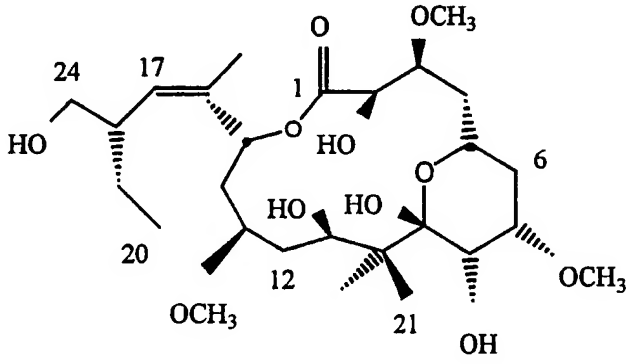
**A. Isolation**

Sponge specimens were collected by SCUBA in Pelorus Sound, South Island, New Zealand at depths of 7-15M. A single frozen specimen (170g wet weight, NIWA # 95DBMYC 2-6) was cut into small segments and extracted with methanol (2 x 600 ml) for 24 hr. The second and first methanolic extracts were passed through a glass column packed with 75 ml of Supelco Diaion HP20® polystyrenedivinylbenzene beads pre-equilibrated with 50% methanol in water. The eluents were combined and passed through the same column. The resulting eluent was diluted with 150 ml of water and passed through the column. Finally the testing eluent was diluted with 2800 ml of water and passed back through the same column. The column was then washed with 100 ml of water and eluted with 150 ml fractions of 1) 20% acetone in water, 2) 55% acetone in water, 3) 55% acetone in 0.2 M $NH_4OH$, and 4) 55% acetone in 0.2 M $NH_4OH$ adjusted to pH 4.0 with AcOH. Fraction 2 was diluted with 150 ml of water and passed through a glass column packed with 35 ml of HP20® pre-equilibrated with water. The column was washed with 50 ml of water and eluted with 100 ml of acetone. The acetone eluent was concentrated to dryness to yield 78.8 mg of a viscous brown oil. The resulting oil was dissolved in 25 ml of methanol and passed through a small glass column containing 250 mg of TosoHass Amberchrom®. The column eluent was diluted with 60 ml of water and passed back through the column. The column was washed with 20 ml of water and the loaded Amberchrom® was transferred on top

of a 20 x 1.5 cm Amberchrom® column pre-equilibrated with water. The column was eluted with increasing concentrations of acetone in water in a stepped gradient fashion. The 32-34% acetone in water fractions were concentrated to dryness to yield a colourless oil (2.2 mg). The 38-40% acetone in water fractions were concentrated to dryness to yield mycalamide A (10.6 mg). The fourth fraction eluted from the original HP20 column at pH 4.0 was diluted with 150 ml of water, adjusted to pH 7.0 with $NH_3$, and passed through a glass column packed with 30 ml of HP20® pre-equilibrated with water. The column was washed with 50 ml of water and eluted with 100 ml of acetone. The acetone eluent was concentrated to dryness to yield 38 mg of a yellow oil. The oil was dissolved in 12 ml of methanol and passed through 2.5 ml of amino bonded phase packing material. The eluent was concentrated to dryness to yield 11.7 mg of pateamine.

**B. Characterisation of the compound present in 32-34% acetone fraction**

The structure of the compound present in the 32-34% acetone fraction was determined to be as follows:




This compound has been termed Peloruside A. The $^1H$ and $^{13}C$ NMR assignments of Peloruside A in $CDCl_3$ are summarised in Table 1 below:

**Table 1. $^{1}H$ and $^{13}C$ NMR Assignments of Peloruside A in $CDCl_3$**

| | $^{13}C$ | | $^{1}H$ | |
|---|---|---|---|---|
| Position | δ (ppm) | mult | δ (ppm) | mult, *J* (Hz) |
| 1 | 173.95 | s | | |
| 2 | 70.26 | d | 4.53 | s |
| 3 | 78.27 | d | 4.22 | dd (10.5, 5.5) |
| 4a | 32.59 | t | 1.78 | M |
| 4b | | | 2.13 | m |
| 5 | 63.51 | d | 4.25 | tdd (11,4.5,2.5) |
| 6a | 31.65 | t | 1.53 | q (12) |
| 6b | | | 1.78 | ddd (12.5,5.5,2.5) |
| 7 | 75.90 | d | 3.82 | ddd (11.5,5,3) |
| 8 | 66.84 | d | 4.02 | d(3) |
| 9 | 101.89 | s | | |
| 10 | 43.63 | s | | |
| 11 | 73.85 | d | 4.89 | br d (10) |
| 12a | 33.93 | t | 1.40 | d (14.5) |
| 12b | | | 2.07 | ddd (15,11.5,4.5) |
| 13 | 77.88 | d | 3.99 | br d (9.5) |
| 14a | 35.68 | t | 2.02 | ddd (15.5, 11.5,1) |
| 14b | | | 2.15 | ddd (15.5, 10.5,1) |
| 15 | 70.86 | d | 5.68 | d (10.5) |
| 16 | 136.05 | s | | |
| 17 | 131.13 | d | 5.05 | d (10) |
| 18 | 43.29 | d | 2.61 | m |
| 19a | 24.60 | t | 1.17 | m |
| 19b | | | 1.44 | m |
| 20 | 12.23 | q | 0.85 | t (7.5) |
| 21 | 15.77 | q | 1.08 | s |
| 22 | 20.77 | q | 1.12 | s |
| 23 | 17.45 | q | 1.67 | d (1) |
| 24a | 66.94 | t | 3.36 | t (10.5) |
| 24b | | | 3.64 | dd (10.5,4) |
| 3Me | 56.09 | q | 3.31 | s |
| 7Me | 55.68 | q | 3.38 | s |
| 13Me | 59.06 | q | 3.48 | s |
| 6OH | | | 6.75 | s |

Figure 1 shows the $^1H$ NMR spectral signature of Peloruside A (300 MHz; pulse sequence : s2pul).

Figure 2 shows the IR spectral signature of Peloruside A.

**C. Bioactivity of Peloruside A**

**Part 1**

The bioactivity of Peloruside A as an anti-tumour agent was determined by the Chemistry Department, University of Canterbury, New Zealand. For the anti-tumour assay a 2-fold dilution series of the sample of interest is incubated for 72 hours with P388 (Murine Leukaemia) cells. The concentration of sample required to reduce the P388 cell growth by 50% (comparative to control cells) is determined using the absorbance values obtained when the yellow dye MTT tetrazollum is reduced by healthy cells to the purple colour MTT formazan. The result is expressed as an $IC_{50}$ in ng/ml.

**Results/Conclusion**

Peloruside A was found to be cytotoxic to P388 murine leukemia cells at approximately 10 ng/ml. Although it bears some structural features of both mycalamide A (*gem*dimethyls and poly hydroxylation) and pateamine (macrolide ring), it is not closely related biochemically.

**Part 2**

**Cytotoxicity Assays**

The cytotoxicity of Peloruside A was tested in five cell lines:

LLC-PK1 (pig kidney)
H441 (human lung adenocarcinoma)
SH-SY5Y (human neuroblastoma)
P388 (murine leukemia)
32D (murine myeloid)

generally in accordance with the MTT assay of Burres *et al.*, *J. Cancer Research* (1989), 49, 2935-2940.

Briefly, cell lines were maintained in Dulbecco's modified Eagle's medium: F12 medium (50:50) (Gibco) supplemented with 10% fetal calf serum (Gibco), 100 mg/ml Penicillin G, and 50 mg/ml streptomycin sulfate. After 96 hours exposure to the toxin, cell viability was determined by the MTT colorimetric assay . MTT standard curves were determined for each cell line, and the MTT absorbance over a range of cell densities was found to be linear for each. Data were analysed with the SYSTAT statistical program using a non-linear model fit, and IC50 values were calculated using a Logit-Log plot.

The following results (expressed as $LD_{50}$ in nM) were obtained:

| Cell Line | LD50 (nM): |
|---|---|
| P388 | 18 |
| H441 | 6.2 |
| LLC-PK1 | 3.7 |
| SH-SY5Y | 14.9 |
| 32D | 7.8. |

**Additional observations**

In cell line H441, the nuclei of the cells were observed to break up into small vessicles (nuclear blebbing). This has not been observed for mycalamide A and pateamine at their respective LD50s.

In cell line SH-SY5Y no retraction of dendrites was observed which contrasts with what has been observed with mycalamide A and pateamine.

In cell line 32D the LD50 was found to increase to 1.6 mM when the cells were assayed for viability at 24 hours of exposure. This dramatic increase in LD50 has not been observed for mycalamide A, pateamine cyclohexamide.

**Conclusion**

These results and observations confirm Peloruside A to be a potent cytotoxin. In particular, the results of the assay conducted in relation to cancerous cell lines are predictive of anti-tumour efficacy in mammals, including humans.

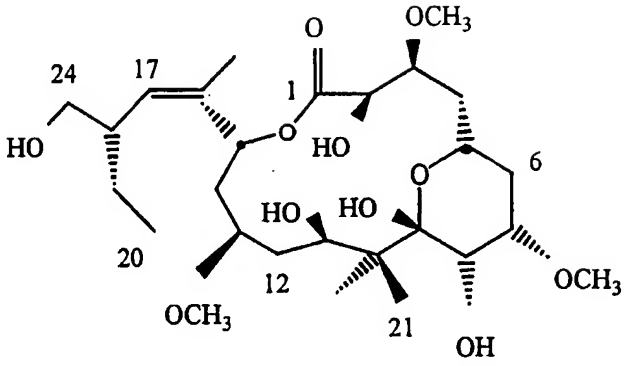
**INDUSTRIAL APPLICATION**

Thus, in accordance with the present invention, the applicants provide a new bioactive compound having cytotoxic properties. This compound can be formulated into medicaments, including pharmaceutical compositions, for use in any prophylactic or therapeutic application for which its cytotoxic properties make it appropriate. Such therapeutic applications include anti-tumour treatment.

Those persons skilled in the art will appreciate that the above description is provided by way of example only and that variations and modifications can be made without departing from the scope of the invention which has been made.

**CLAIMS:**

1. A compound of formula (I)




or a functionally equivalent analogue thereof.

2. A bioactive compound which has the NMR and/or IR spectral signature of Figures 1 and 2.

3. A composition which comprises a compound as defined in claim 1 or claim 2, together with a suitable carrier therefor.

4. A pharmaceutical composition which comprises a compound as defined in claim 1 or claim 2, together with a pharmaceutically-acceptable carrier therefor.

5. A pharmaceutical composition as defined in claim 4 in which said compound is a compound of formula (I) as defined in claim 1.

6. The use of a compound as defined in claim 1 or claim 2 in the preparation of a medicament.

7. The use of claim 6 wherein said medicament is a cytotoxic medicament.

8. The use of claim 6 wherein said medicament is an anti-tumour medicament.

9. A method of prophylaxis or therapy which comprises the step of administering to a patient in need of the same a bioactive compound as defined in claim 1 or claim 2 or a pharmaceutical composition as defined in claim 4 or claim 5.

10. A method as defined in claim 9 which is, or is part of, a treatment against cancer.

1/2

FIGURE 1

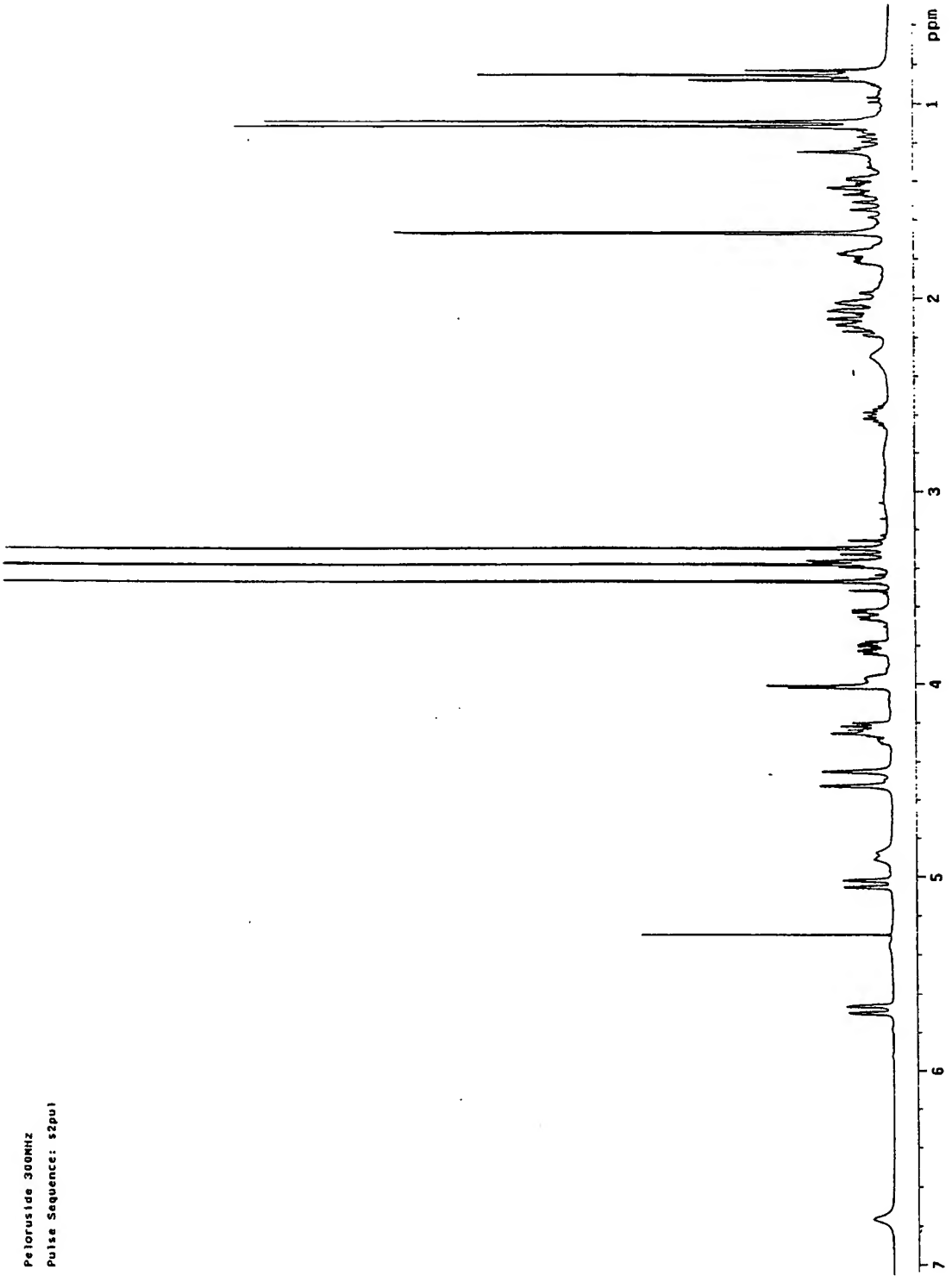


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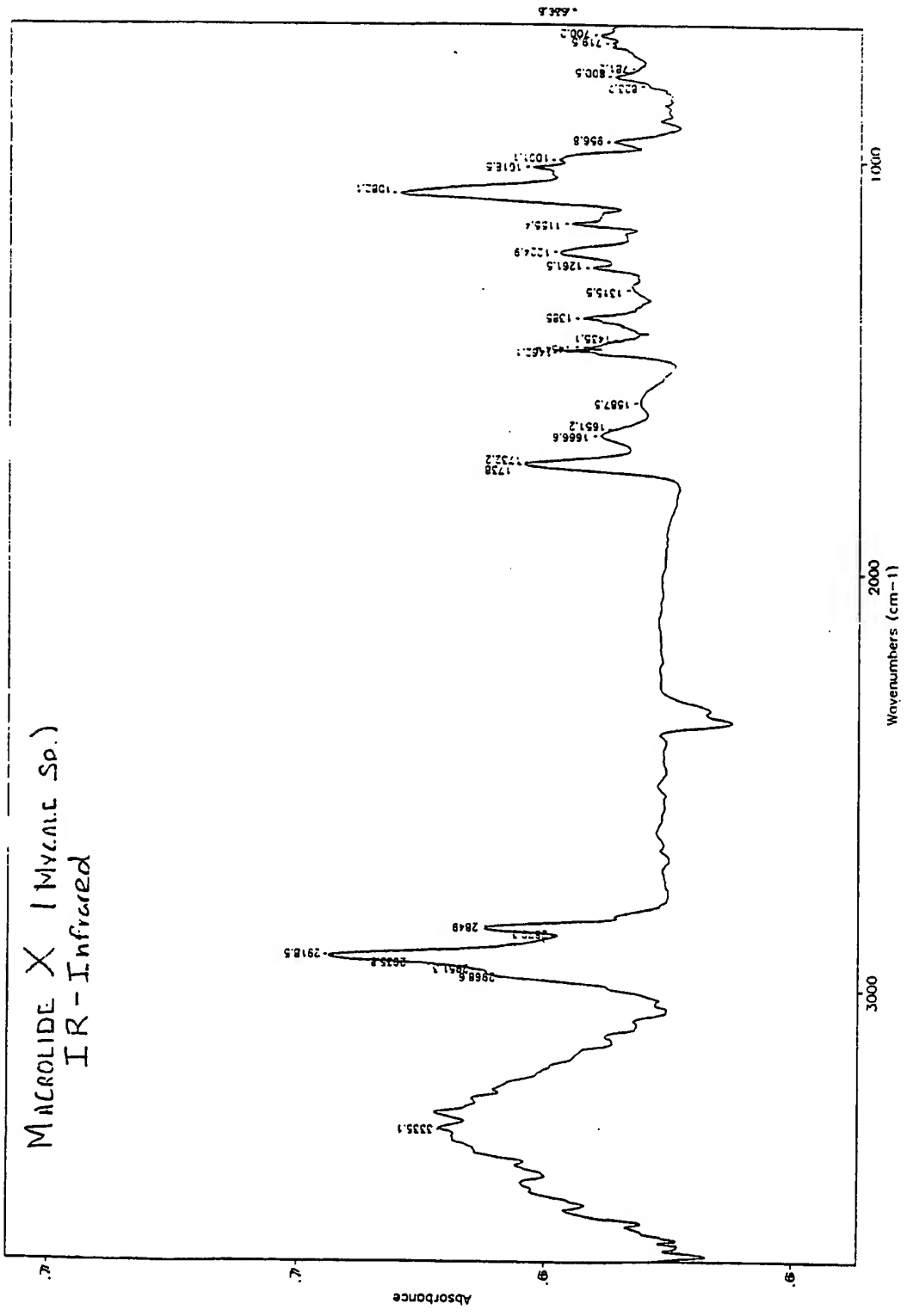
FIGURE 2


MACROLIDE X ( MYCALE SP.)
IR - Infrared
Absorbance
Wavenumbers (cm-1)
3000
2000
1000
3335.1
2918.5
2849
1738
1732.2
1666.6
1587.5
1455.1
1435.1
1385
1315.5
1261.5
1224.9
1155.4
1085.1
1016.5
956.8
798.2
719.5
700.2
646.5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NZ00/00152

**A. CLASSIFICATION OF SUBJECT MATTER**

Int. Cl. [7]: C07D 493/06; A61K 31/355

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS OnLine: molecular formula of claim 1

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| P, X | Chemical Abstracts, abstract no. 132:149195, 2000; West L M et al: "Peloruside A: A potent cytoxic macrolide isolated for the New Zealand marine sponge Mycale sp." [J.Org. Chem. (2000), 65(2), 445-449] See abstract and particularly Chemical Abstracts Registry No. 257939-61-0 | 1-10 |

☐ Further documents are listed in the continuation of Box C ☐ See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 27 November 2000 | 4 - DEC 2000 |

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